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TI The search for novel adjuvants for early life vaccinations: can "danger" motifs show us the way?.
AU Kovarik J; Siegrist C A
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Immunostimulatory sequence oligodeoxynucleotide-based vaccination and immunomodulation: Two unique but complementary strategies for the treatment of allergic diseases

Anthony Adam Horner, MD, and Eyal Raz, MD *La Jolla, Calif*

Despite a number of effective pharmaceutical options for the prevention and treatment of the pathophysiologic responses that occur in sensitized patients on allergen exposure, the termination of allergic hypersensitivities remains an elusive therapeutic goal. Traditional immunotherapy with allergen extracts is the only currently used intervention that has been shown to induce allergen tolerance, but it has a limited scope of efficacy. However, recent studies suggest that immunostimulatory sequence oligodeoxynucleotide (ISS-ODN)–based interventions might offer an alternative and potentially more effective means for extinguishing T_H2-biased hypersensitivities. Three basic ISS-ODN–based immunotherapeutic strategies have been studied to date. Immunization with allergen mixed with ISS-ODN, immunization with allergen–ISS-ODN conjugates, and immunomodulation with ISS-ODN alone all have proved efficacy in the attenuation of the allergic phenotype in mice. Preliminary results with allergen–ISS-ODN conjugate vaccines in allergic patients have also been encouraging. This article will provide our perspective on the application of ISS-ODN–based vaccination and immunomodulation to the treatment of atopic diseases and the immunologic basis for their antiallergic activities. (J Allergy Clin Immunol 2002;110:706-12.)

Key words: Immunostimulatory DNA, cytosine-phosphate-guanine motif, immunotherapy, immunomodulation

Abbreviations used

AIC: Allergen physically conjugated to ISS-ODN
APC: Antigen-presenting cell
CpG: Cytosine-phosphate-guanine
ISS-ODN: Immunostimulatory sequence ODN
NK: Natural killer
ODN: Oligodeoxynucleotide
OIC: OVA conjugated to ISS-ODN
OIC-H: OVA/ISS-ODN conjugation ratio of 1:6
OIC-L: OVA/ISS-ODN conjugation ratio of 1:3
OVA: Ovalbumin
OVA–M-ODN: OVA conjugated to a mutated control ODN
OVA–M-ODN-M: OVA conjugated to mutated ODN at a 1:4 molar ratio

PRESENT THERAPEUTIC OPTIONS FOR THE TREATMENT OF ALLERGIC DISEASE

Allergic diseases affect as many as 30% of individuals in some populations, and incidence and prevalence rates continue to increase.[1] Paradoxically, these trends are occurring at a time when our understanding of the pathophysiologic mechanisms of atopy are fairly well developed. Furthermore, a large number of effective therapies are currently available for the treatment of allergic end organ pathology, such as corticosteroids for the reversal of allergic inflammation, antihistamines and leukotrienes for the attenuation of pathology associated with mast cell and eosinophil degranulation, and β_2-receptor agonists for the reversal of bronchospasm.[2,3] However, none of these medications have been shown to reverse the underlying allergen hypersensitivities that perpetuate the allergic phenotype. Desensitization can be achieved with traditional protein-based immunotherapy. However, allergen immunotherapy (1) requires repeated injections, (2) takes several months to have a therapeutic effect, (3) is generally less effective than pharmaceutical interventions for the treatment of respiratory allergic symptoms, (4) is ineffective and unsafe for the treatment of food allergies, and (5) has associated risks, such as life-threatening anaphylaxis.[4-7] Thus there is a clear and present need for more effective strategies to prevent and reverse the T_H2-biased immune dysregulation that fuels the pathogenesis of allergic conditions.

In contrast to the limitations of current therapies, several immunostimulatory oligodeoxynucleotide (ISS-ODN)–based therapeutic strategies have proved highly effective for the prevention and reversal of T_H2-mediated

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hypersensitivity states in mouse models of allergic disease,[8] and phase I clinical trial data have also been encouraging.[9,10] Such results have generated guarded optimism that ISS-ODN–based immun therapeutics could prove to be a silver bullet for the future treatment of allergic diseases and for the reversal of current trends toward their ever increasing prevalence.

FROM GENE VACCINES TO ISS-ODN–BASED IMMUNOTHERAPEUTICS

The observation that plasmid gene vaccination induced a highly T_H1-biased immune response and prevented the development of T_H2-biased immunity was first published in 1996.[11] Compared with mice injected with protein in alum, mice injected with plasmid encoding the gene product were shown to develop high IgG2a (IFN-γ dependent) and low IgG1 and IgE (IL-4 dependent) responses. In addition, gene-vaccinated mice had a T_H1-biased cytokine profile characterized by high IFN-γ and low IL-5 production. Furthermore, gene vaccination was shown to prevent the development of T_H2-biased immunity and to reverse preexisting T_H2-biased immune profiles. Initial studies used experimental allergens, such as β-galactosidase and ovalbumin (OVA), and more recently have been replicated with clinically relevant allergens, such as house dust mite, birch, latex, and peanut.[8]

In the course of evaluating the immunogenicity of various gene vaccination vectors, it was discovered that gene expression was one of 2 factors that determined their immunogenicity. Work from several laboratories demonstrated that effective gene vaccination vectors additionally contained cryptic immunostimulatory DNA sequences (ISS-ODN or cytosine-phosphate-guanine [CpG] motifs) that provided T_H1 adjuvant activity for the immune responses that developed toward their gene product.[12,13] Deletion of several of these ISS-ODNs from the vaccination plasmid attenuated the postvaccination immune response without affecting gene expression, and the immune response was restored by means of covaccination with a plasmid containing additional ISS-ODN.

ISS-ODNs were first described in Freund adjuvant, an emulsion of mycobacterial extract in paraffin oil that has been used to improve vaccine responses for more than 60 years.[14,15] Tokunaga et al[15,16] initially published that purified DNA from *Mycobacterium bovis* BCG induced limited antitumor activity, natural killer (NK) cell activation, and IFN-α/β production but that these activities were lost with DNAse treatment.[15,16] In murine studies ISS-ODNs with optimal activity were found to contain unmethylated CpG dinucleotides within a palindromic hexamer that follows the following formula: 5′-purine-purine-CpG-pyrimidine-pyrimidine-3′ (eg, 5′-GpApCpGpTpC-3′, 5′-ApGpCpGpCpT-3′, and 5′-ApApCpGpTpT-3′).[17] However, subsequent human studies suggest that the thymidine-phosphate-cytosine-phosphate-guanine trinucleotide might be more important than the CpG nucleotide for optimal activation of human mononuclear cells by ISS-ODN.[18,19]

Interestingly, CpG dinucleotides generally occur at near the expected frequency (1 in 16) in many bacterial genomes but are much less frequent in mammalian DNA. In addition, less than 5% of the cytosines in CpG dinucleotides of bacterial genomes are methylated, whereas 70% to 90% of the CpG dinucleotides in mammalian genomes contain methylated cytosines.[20] We and others have shown that cytosine methylation neutralizes the immunologic activity of CpG motifs in bacterial DNA and synthetic ISS-ODN.[21] These and other observations suggest that the capacity to detect unmethylated CpG (TpCpG) motifs provides for innate host recognition of microbial DNA and immunologic protection against infection by potential pathogens.

Studies with synthetic phosphorothioate ISS-ODNs have shown that they elicit a robust and multifaceted innate immune response in mice analogous to the immune response elicited with bacterial DNA.[21-24] The innate response to ISS-ODN is characterized by the production of IL-12, IL-18, IFNs (α, β, and γ), IL-6, and IL-10 and the upregulation of costimulatory molecules by antigen-presenting cells (APCs), B cells, NK cells, and potentially other cell types. However, although allergic effector cells, such as mast cells and eosinophils, have not been adequately evaluated, T cells are known to have a poor direct response to ISS-ODN, and they do not express the ISS-ODN receptor (toll-like receptor 9) at significant levels.[25] Functionally, ISS-ODN increases NK cell activity, matures APCs to elicit T-cell responses, and induces B-cell proliferation and antibody production.[21,23,26] Mammalian DNA, methylated bacterial DNA, and methylated ISS-ODN, in contrast, do not induce these responses.[23]

In a number of studies, ISS-ODN has further proved to be an effective T_H1 and mucosa adjuvant, generating immune responses to coadministered protein antigens that mimic the immune responses seen after gene vaccination.[27-29] Additional studies have demonstrated that the T_H1-biased immune profile elicited with protein–ISS-ODN vaccination is resistant to subsequent perturbation toward a T_H2-biased phenotype.[27,30,31] Furthermore, ISS-ODN has a long-lasting T_H1-biasing adjuvant effect on antigens (and potentially allergens) encountered at the site of delivery.[32,33] In fact, ISS-ODN delivery 1 to 7 days before antigen (prepriming) leads to immune responses of greater magnitude then those seen with antigen–ISS-ODN codelivery. The prepriming activity of ISS-ODN is likely to be due to its ability to activate mononuclear cells for 2 weeks or more in vivo.[26,32] The robust and persistent immune activation and T_H1 adjuvant activity elicited by ISS-ODN has prompted its study as an antiallergic vaccine adjuvant and immunomodulator. These studies will be reviewed and interpreted in the text to follow.

ISS-ODN–BASED VACCINATION FOR THE TREATMENT OF ALLERGIC DISEASES

Traditional allergen immunotherapy is based on the principles of vaccination against infectious agents. The therapeutic goal is the induction of a protective immune

TABLE I. OIC-L and OIC-H are more immunogenic than OVA plus ISS-ODN

Immunization	IgG2a (U/mL)	IgG1a (U/mL)	IFN-γ (pg/mL)	IL-10 (pg/mL)
OVA	193 ± 79	1020 ± 608	194 ± 75	<20
OVA + ISS-ODN	596 ± 113	2330 ± 743	409 ± 98	<20
OIC-L	8124 ± 1408*	2013 ± 1238	1385 ± 220*	748 ± 543*
OIC-H	4840 ± 2075*	1157 ± 379	989 ± 151*	378 ± 143*
OVA–M-ODN-M	194 ± 80	1264 ± 428	89 ± 47	<20

Mice received a single intradermal immunization with OVA, OVA plus ISS-ODN, or ODN-conjugated OVA. Free ISS-ODN was delivered at a dose corresponding to the ISS-ODN content of OIC-H. Antigen-specific serum antibody and splenocyte cytokine responses are presented as means for 4 mice per group ± SE (*$P < .001$ relative to OVA vaccination). These data have been previously published.[36]

TABLE II. OVA-ODN conjugates are less anaphylactogenic than native OVA

Challenge	Histamine (μmol/L)	Survival (%)
Saline	ND	6/6 (100%)*
OVA	28 ± 2	0/14 (0%)
OVA + ISS-ODN	27 ± 1	0/10 (0%)
OIC-L	15 ± 6†	10/12 (83%)*
OIC-H	2 ± 1‡	10/10 (100%)*
OVA–M-ODN-M	12 ± 1†	9/10 (90%)*

Mice were T_H2 sensitized with OVA and alum and then were intravenously challenged with OVA, OVA plus ISS-ODN, OIC-L, OIC-H, or OVA–M-ODN-M. Challenge doses were calculated as discussed in Table I. Histamine determinations were conducted with plasma obtained 2 minutes after allergen challenge (means from 4 mice per group ± SE). Lethal anaphylaxis occurred within 1 hour of challenge. These data have been previously published.[36]

ND, None detected.

*$P < .001$ relative to OVA challenge.

†$P < .05$ relative to OVA challenge.

‡$P < .05$ compared with OIC-L challenge.

response to an allergen to which a clinical hypersensitivity preexists by affecting a change in the allergen-specific adaptive immune repertoire. In this vein vaccination with allergen mixed with ISS-ODN has proved significantly more effective than vaccination with allergen alone in the induction of T_H1-biased and the reversal of T_H2-biased immune profiles.[27,30,34] Moreover, ISS-ODN–based vaccines induce an allergen-specific IL-10 response.[35,36] However, although an IL-10 response has been associated with clinically effective immunotherapy, IL-10 is not traditionally considered to be a T_H1 cytokine.[37] In association with the prevention and reversal of allergic (T_H2 biased) immune profiles, ISS-ODN–based vaccines have been further shown to protect against the immediate hypersensitivity response of anaphylaxis and the late-phase allergic response of asthma.[8,34] In contrast, control mice immunized with allergen alone were not protected from T_H2-biased immune deviation or from the development of allergic hypersensitivity responses in these studies.

Physical conjugates of allergen and ISS-ODN (AIC) were developed because it was believed that AIC might be more immunogenic and less allergenic than allergen mixed with ISS-ODN. From the standpoint of immunogenicity, conjugation of adjuvant to allergen was thought to improve their colocalization, leading to antigen presentation on ISS-ODN–activated APCs. Another rationale for the improved immunogenicity of AIC was provided when oligodeoxynucleotide (ODN) conjugation was shown to facilitate APC uptake and presentation of antigens.[38] From the perspective of allergenicity, it was speculated that conjugation of negatively charged ODNs to allergen would mask Ig-binding epitopes, thereby reducing their capacity to induce IgE-mediated hypersensitivity responses in the allergic host.

The improved immunogenicity and reduced allergenicity of AIC compared with allergen–ISS-ODN mixtures have now been demonstrated by several groups.[35,36,38-41] Results presented in Table I highlight the improved immunogenicity of AIC compared with that of allergen mixed with ISS-ODN.[36] For these investigations, we synthesized OVA conjugated to ISS-ODN (OIC) with OVA–ISS-ODN conjugation ratios of 1:3 (low; OIC-L) and 1:6 (high; OIC-H) and a control conjugate of OVA linked to a mutated ODN (OVA–M-ODN) at a 1:4 molar ratio (medium; OVA–M-ODN-M). With a single immunization, both OIC-L and OIC-H were significantly more immunogenic than OVA mixed with ISS-ODN, inducing stronger T_H1-biased antibody and cytokine responses and a robust IL-10 response not seen in other immunization groups. In contrast, OVA–M-ODN-M vaccination led to a weak immune response similar in magnitude and T_H bias to that induced by means of vaccination with native OVA alone.

We have also recently observed that OIC was significantly less allergenic than native allergen in several models of Ig-dependent hypersensitivity, including a model of anaphylaxis.[36] In the anaphylaxis model (Table II) intravenous OVA challenge of OVA-sensitized mice led to 0% survival, whereas challenge with equivalent doses of OIC-L, OVA–M-ODN-M, and OIC-H (on the basis of OVA content) led to 83%, 90%, and 100% survival, respectively, with postchallenge plasma histamine levels mirroring anaphylactic challenge survival data. Additional in vitro studies with OVA-IgE–armed bone marrow–derived mast cells demonstrated a stepwise decrease in degranulation, with OVA challenge greater than OIC-L challenge greater than OVA–M-ODN-M challenge greater than OIC-H challenge, and OVA was more than 100-fold more allergenic than OIC-H.[36]

Considered in conjunction with other studies, data presented in Table I demonstrate that the improved

immunogenicity of AIC over native allergen and allergen mixed with ISS-ODN is dependent on both the intrinsic immunostimulatory activity of the ISS-ODN sequence and its conjugation to allergen.[36,38,40,41] In contrast, results presented in Table II demonstrate that the reduced allergenic potential f AIC compared with native allergen and allergen mixed with ISS-ODN is a function of the allergen/ODN conjugation ratio but is not dependent on the immunostimulatory activities of ISS-ODN because OVA–M-ODN-M was also significantly less allergenic than native protein.[36] Preliminary results from several phase I clinical trials have been consistent with results from our murine studies, suggesting that AIC might also prove to be safer and more effective than traditional allergen extracts for immunotherapy in allergic patients.[9,10]

ISS-ODN–BASED IMMUNOMODULATION FOR THE TREATMENT OF ALLERGIC DISEASES

ISS-ODN–based vaccines used according to the principles of vaccination against infectious agents have proved highly effective for the induction of T_H1-biased immune responses and the prevention of T_H2-biased immune deviation.[8] However, observations first made in collaboration with David Broide in 1998 led us to suggest an alternative paradigm for the use of ISS-ODN in the treatment of allergic diseases.[42] We found that allergen-independent delivery of ISS-ODN, either by means of injection or the intranasal route, provided almost immediate protection against asthma in T_H2-sensitized mice. This allergen-independent immunomodulatory activity of ISS-ODN has since been observed by other investigators.[43-45] In fact, ISS-ODN immunomodulation has proved more effective than corticosteroid therapy in the prevention of shock organ pathology in murine models of allergic conjunctivitis and asthma.[42-44,46] Moreover, like corticosteroids, allergen-independent ISS-ODN delivery rapidly inhibits allergen-specific IL-5 production from mononuclear cells isolated from T_H2-sensitized mice. However, in contrast to corticosteroids, ISS-ODN immunomodulation also induces allergen-specific IFN-γ production from these T_H2-sensitized mononuclear cells.

In vitro studies demonstrate that ISS-ODN also functions as an immunomodulator for mononuclear cells isolated from atopic patients. Consistent with previous murine studies, in these investigations ISS-ODN elicited the allergen-independent production of cytokines (ie, IL-12, IFN-α, IFN-γ, and IL-10) known to inhibit allergic immune deviation and effector cell (ie, T_H2 cell, eosinophil, and mast cell) responses.[18,47-49] In addition, ISS-ODN increased IFN-γ receptor (CD119) and decreased IL-4 receptor (CD124) expression on B cells derived from allergic patients.[18] Furthermore, ISS-ODN inhibited IL-4–dependent IgE synthesis from atopic PBMCs in an IL-12–, IFN-α–, IFN-γ–, and IL-10–dependent manner.[18,48] In studies with precommitted human T_H2 cells, ISS-ODN has also been shown to skew their cytokine profile to a more T_H1-biased phenotype by means of IFN- and IL-12–dependent mechanisms.[50] Taken together with the results of murine studies, these human studies establish that ISS-ODN inhibits allergic immune dysregulation, at least in part by inducing the allergen-independent production of antiallergic cytokines from APCs, B cells, and NK cells. Furthermore, ISS-ODN regulates cytokine receptor expression in a manner that is likely to favor responsiveness to IFN-γ over IL-4. Along with providing a framework for understanding the intrinsic antiallergic immunomodulatory activities of ISS-ODN, these results provide a rationale for conducting future clinical trials of ISS-ODN–based immunomodulation with allergic patients.

COMPARING ISS-ODN–BASED VACCINATION AND ISS-ODN IMMUNOMODULATION

The principles of vaccination and immunomodulation, as discussed herein, represent 2 distinct yet interrelated strategies for the use of ISS-ODN–based reagents in the treatment of allergic diseases. In further consideration of the dichotomy between ISS-ODN–based vaccination and immunomodulation, we have found that ISS-ODN–containing vaccines induce antigen-specific and T_H1-biased immune responses that are maintained for at least 1 year and are resistant to T_H2-biased immune deviation for at least 5 months,[40,41] whereas allergen-independent immunomodulation with ISS-ODN protects against the allergic phenotype for a much shorter period of time.[46] For example, in experiments discussed previously, a single dose of ISS-ODN given shortly before airway allergen challenge of T_H2-sensitized mice led to a dramatic attenuation of their asthmatic response to airway allergen challenge and inhibition of allergen-specific IL-5 production, whereas allergen-specific IFN-γ production increased in these mice. In subsequent studies we found that these mice were protected from a second allergen challenge for an additional month. However, 2 months after ISS-ODN delivery and primary airway allergen challenge, responses to a second airway challenge and the T_H bias of the immune profiles of these mice were similar to those of T_H2-sensitized mice that did not receive ISS-ODN before primary airway allergen challenge.[46]

Another point of divergence between the paradigms of ISS-ODN–based vaccination and immunomodulation is that ISS-ODN–based vaccines induce the expansion of allergen-specific and protective clones of T and B cells over weeks.[41] In contrast, immunomodulation by ISS-ODN is allergen independent, and protection from allergic hypersensitivity responses develops within hours of ISS-ODN delivery. In this vein allergen-independent ISS-ODN immunomodulation has been shown to attenuate the asthmatic response of allergic mice not only when given before challenge but also when given during or even after allergen challenge.[45,51] Therefore it is unlikely that ISS-ODN–based immunomodulation directly attenuates allergen challenge responses at the level of adaptive immunity. We consider it far more reasonable to suggest that innate responses to ISS-ODN by APCs, B

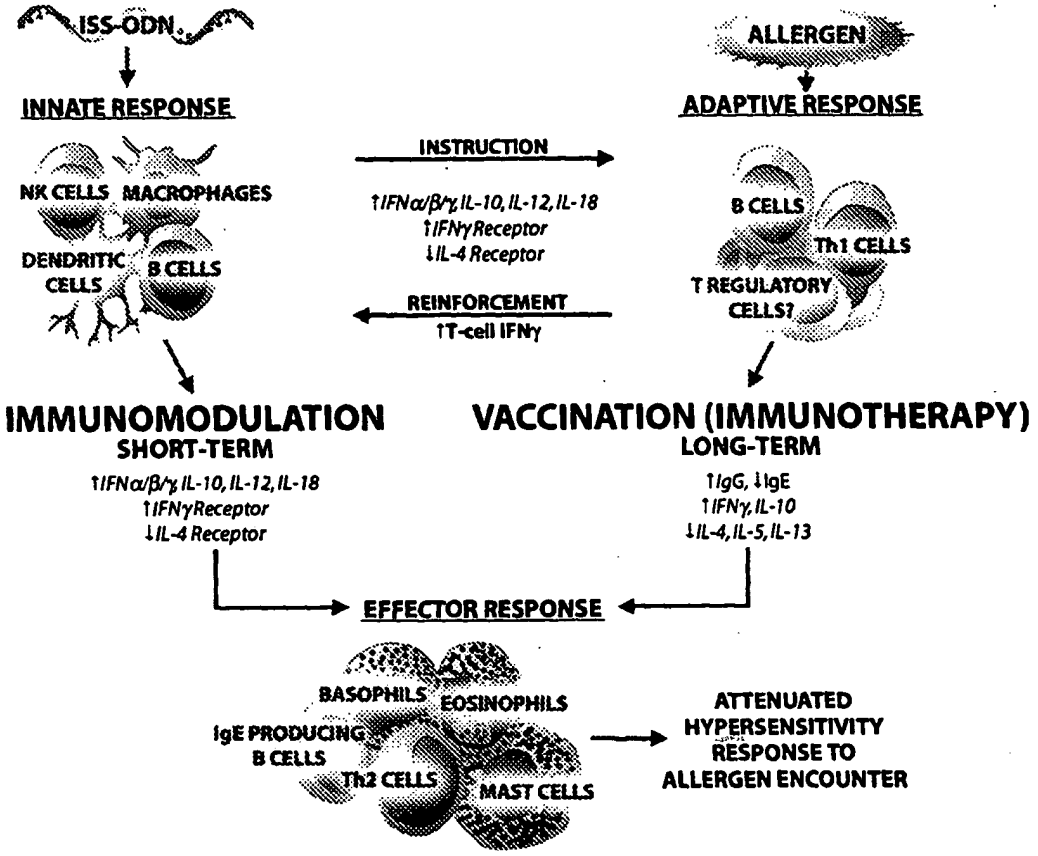

FIG 1. Dichotomy between ISS-ODN–based immunomodulation and vaccination. ISS-ODN activates innate immunity, leading to the production of antiallergic and T_H1-promoting cytokines while increasing IFN-γ receptor and reducing IL-4 receptor expression on responding cells. As a consequence, ISS-ODN immunomodulation rapidly inhibits the effector arm of the allergic hypersensitivity response (ie, precommitted T_H2 cells, IgE-secreting B cells, eosinophils, and mast cells) in an allergen-independent but time-limited manner (4-6 weeks). ISS-ODN–induced innate immune activation also affects adaptive immunity, promoting T_H1-biased and inhibiting T_H2-biased responses to encountered allergens while stimulating an allergen-specific IL-10 response. In contrast to ISS-ODN–based immunomodulation, adaptive immune responses to ISS-ODN–based vaccines develop slowly, are allergen specific, and are long lived (≥1 year). Thus ISS-ODN–induced activation of 2 separate but complimentary arms of the immune system (innate and adaptive) provides both rapid and long-lasting protection from T_H2-biased immune deviation and allergic diseases.

cells, and NK cells (eg, type 1 IFNs, IFN-γ, IL-12, and IL-10 production and modulation of cytokine receptor expression) indirectly lead to time-limited inhibition of the functions of allergic effector cells (eg, allergen-specific T_H2 cells and IgE-producing B cells, as well as mast cells and eosinophils). We further speculate that although B- and T-cell memory are not irreversibly affected by ISS-ODN immunomodulation, repeated allergen presentation by ISS-ODN–activated APCs would imprint a long-term antiallergic T_H bias on allergen-specific immune memory by committing increasing numbers of B and T cells with each successive allergen encounter. In Fig 1 we propose our schema for how ISS-ODN effects on innate immunity (immunomodulation) and adaptive immunity (vaccination) contribute to the prevention and reversal of allergic immune deviation and disease.

CONCLUSION

Over the past decade, a growing body of experimental evidence suggests that ISS-ODN–based immunotherapeutics might be effective in the treatment of allergic diseases and the reversal of the allergen-specific hypersensitivities that mediate them.[8] ISS-ODN–based reagents have been shown to be effective in the prevention and treatment of allergic diseases when used according to the principles of immunization, and AIC has proved to be a particularly attractive immunization reagent because of its improved immunogenicity and reduced allergenicity compared with that of allergen mixed with ISS-ODN. Previous studies have made it clear that ISS-ODN–based vaccines elicit highly T_H1-biased immune responses and an associated IL-10 response. However, given that both allergen-specif-

ic IFN-γ and IL-10 responses have been reported to inhibit the allergic phenotype in patients receiving traditional immunotherapy,[37,52] the roles these cytokines play in protecting mice that receive ISS-ODN–based vaccines remain to be determined. In this vein a family of T-regulatory cells producing relatively low amounts of proinflammatory cytokines and high amounts of IL-10 have recently been proposed to inhibit several diseases of immune dysregulation, including allergic diseases.[53,54]

In addition to being a potent antiallergic vaccine adjuvant, ISS-ODN has proved to have utility as an immunomodulator when used independently of allergen, much in the way that corticosteroids are presently used to treat allergic diseases.[8] Nonetheless, although their antiallergic activities might appear similar, ISS-ODN–based vaccines offer long-lasting but allergen-specific protection, whereas ISS-ODN–induced immunomodulation is allergen independent but time limited. However, if used in clinical practice, ISS-ODN immunomodulation has the practical advantage that a patient's specific allergic hypersensitivities might not need identification.

Clearly, many questions about the mechanisms by which ISS-ODN–based immunotherapeutics protect against the allergic phenotype remain. However, as schematically represented in Fig 1, we believe that the antiallergic effects of ISS-ODN on innate (allergen nonspecific) and adaptive (allergen specific) immunity should be considered as distinct but interrelated phenomena. By extrapolation, we further propose that ISS-ODN–based immunomodulation and vaccination represent 2 unique but complementary strategies for the treatment of allergic diseases.

Phase 1 AIC vaccination trials with Amb a 1–ISS-ODN conjugate (Amb a 1 is the major ragweed allergen) in ragweed-sensitive patients with allergic rhinitis have already been conducted, and future trials of ISS-ODN–based immunomodulation are anticipated. Preliminary results from the Amb a 1–ISS-ODN conjugate trials have shown that with as few as 6 injections, Amb a 1–ISS-ODN conjugate reduced allergic rhinitis symptoms and need for rescue medications during the ragweed season.[9] Furthermore, Amb a 1–ISS-ODN conjugate elicited few allergic reactions even when used at doses considered unsafe with traditional allergen immunotherapy extracts.[9,10] If ISS-ODN–based immunotherapeutics continue to demonstrate the high levels of safety and efficacy in allergic patients that have been seen in preclinical animal studies and phase 1 clinical trials, they will likely revolutionize our current approach to the treatment of allergic diseases.

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